 Human Molecular Genetics, 1997, Vol. 6, No. 6 943–947

An insertion mutation of the CHRNA4 gene in a family with autosomal dominant nocturnal frontal lobe epilepsy

Ortrud K. Steinlein[1,*], Andres Magnusson[2,3], Jens Stoodt[1], Sonia Bertrand[4], Sigrid Weiland[1], Samuel F. Berkovic[5], Karl O. Nakken[3], Peter Propping[1] and Daniel Bertrand[4]

[1]Institute for Human Genetics, University of Bonn, Wilhelmstrasse 31, 53111 Bonn, Germany, [2]Department of Psychiatry, Ullvaal Hospital, Olso, Norway, [3]National Center for Epilepsy, Sandvika, Norway, [4]Department of Physiology, Medical Faculty, Geneva, Switzerland and [5]Department of Medicine (Neurology), University of Melbourne, Melbourne, Australia

Received January 20, 1997; Revised and Accepted March 24, 1997

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is the first, and to date only, idiopathic epilepsy for which a specific mutation has been found. A missense mutation in the critical M2 domain of the α4 subunit of the neuronal nicotinic acetylcholine receptor (CHRNA4) has been recently identified in one large Australian pedigree. Here we describe a novel mutation in the M2 domain of the CHRNA4 gene in a Norwegian family. Three nucleotides (GCT) were inserted at nucleotide position 776 into the coding region for the C-terminal end of the M2 domain. Physiological investigations of the receptor reconstituted with the mutated CHRNA4 subunit reveal that this insertion does not prevent the receptor function but increases its apparent affinity for ACh. In addition, this mutant receptor shows a significantly lower calcium permeability that, at the cellular level, may correspond to a loss of function. Comparison of the two mutations identified so far in families with ADNFLE illustrates that different mutations can result in similar phenotypes.

INTRODUCTION

About 40% of all epilepsies are idiopathic. Although it has long been known that genetic factors play a major role in the aetiology of idiopathic epilepsies, the molecular mechanisms are largely unknown. Familial clustering of common generalised idiopathic epilepsy subtypes suggests a multiplicative contribution of several loci. In contrast to the common idiopathic epilepsies, like childhood absence epilepsy, juvenile absence epilepsy, or juvenile myoclonic epilepsy, some rare focal and generalised forms are inherited as monogenic traits.

Autosomal dominant nocturnal frontal epilepsy (ADNFLE) is a recently recognised disorder characterized by clusters of brief motor seizures, mostly arising from sleep. Clinical onset is usually in childhood and the disorder often persists throughout adult life. The penetrance of the mutant gene is ~70%. Misdiagnosis of this treatable epilepsy as normal sleep behaviour, parasomnias or psychiatric disturbance is common (1,2). The interictal EEG is usually normal and even ictal recordings do not always show definitive epileptiform activity (2,3).

There is evidence that ADNFLE can be due to dysfunction of neuronal nicotinic acetylcholine receptors (nAChR). In one large Australian pedigree with ADNFLE the disorder was mapped to 20q13.2–q13.3 (4). The responsible mutation was subsequently identified as a missense mutation which replaces a serine into phenylalanine (Ser248Phe) in the α4 subunit gene (CHRNA4) of the neuronal nAChR (5). This mutation affects the second transmembrane domain (M2) which has been shown to form the wall of the ion channel. This has been the first, and to date only, mutation described in an idiopathic epilepsy.

Since the description of ADNFLE in 1994, many other families have been recognised (3,6). Until now neither the Ser248Phe mutation, nor any other CHRNA4 mutation has been found in those families (unpublished data). Here we describe a novel CHRNA4 mutation in ADNFLE, observed in a Norwegian family (Fig. 1), which supports the etiological role of CHRNA4 in this epilepsy.

Reconstitution experiments performed in *Xenopus* oocytes with either the normal α4β2 nAChR or the mutant α4(S248F)β2 or α4(776ins3)β2 nAChRs demonstrate that these two genetic alterations induce distinct modifications of the receptor's functional properties. Hypotheses based on these observations are discussed in the light of the high calcium permeability displayed by the neuronal nAChRs.

RESULTS

The entire coding region of the CHRNA4 gene was amplified from the DNA of the index patient (family member IV-2; Fig. 1), followed by analysis of the PCR fragments by SSCA and

*To whom correspondence should be addressed. Tel: +49 228 287 2568; Fax: +49 228 287 2380; Email: human@snphysio2.wilhelm.uni-bonn.de

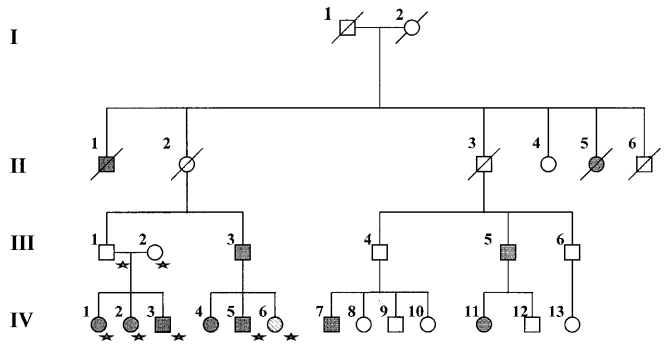

Figure 1. Pedigree of the Norwegian family with autosomal dominant nocturnal frontal lobe epilepsy. Family members available for molecular analysis are marked by an asterisk. Filled symbols indicate family members who have been diagnosed with ADNFLE. IV6 (hatched symbol) is possibly affected (see text).

heteroduplex analysis. PCR fragments amplified with primers 5′-CCT GCC CTC CGA GTG TGG C-3′ and 5′-GGG CAT GGT GTG CGT GCG TG-3′ revealed clearly abnormal migration of both single-stranded and double-stranded DNAs. The PCR product was sequenced both directly and after subcloning (Fig. 2) and the results indicated an insertion of three nucleotides (GCT) following bp776 [referring to *Torpedo* α subunit gene numbering (7); bp788 according to human α4 subunit gene numbering (8)]. The insertion does not alter the open reading frame but results in the insertion of an additional leucine residue near the C-terminal end of the second transmembrane domain (Fig. 3). The 776ins3 mutation (nomenclature according to ref. 9) was found in family members III-1, III-3, IV-1, IV-2, IV-3, IV-5 and IV-6, too, but not in III-2 nor in a control panel made up of 254 independent Caucasian blood donors.

Mutant cDNA reconstitute functional nAChR

It is well documented that functional nAChR resembling the major brain receptor can be reconstituted in a host system by expression of at least the α4 and β2 subunits (10–14). Therefore, in an attempt to assess the effects of the leucine 776 insertion found in the Norwegian family on the receptor properties, reconstitution experiments were designed using co-expression of the control (normal α4β2 nAChR; CT) or mutated α4 subunit [α4(776ins3)] in combination with the control β2 subunit.

Oocytes injected with the cDNAs coding for the control α4 and β2 subunits showed robust inward currents in response to acetylcholine (ACh) application (Fig. 4A, upper panel). Currents evoked by short ACh pulses at saturating concentration typically ranged within 2–10 μA. As expected from previous descriptions of α4β2 nAChRs these currents displayed little or no desensitization over a 2 s agonist application. A plot of the peak currents evoked by ACh as a function of the agonist concentration yielded a classical dose–response curve that is adequately described by the empirical Hill equation (squares, Fig. 4B).

When the same experiments are performed with the α4(776ins3) and β2 subunits in sibling oocytes a large current is also observed in response to ACh (Fig. 4A, lower panel). Currents evoked by saturating ACh concentrations had comparable amplitudes to those of the control. Moreover, no differences could be observed on the time course of the ACh-evoked responses. In contrast, however, the mutant receptor is ~10-fold more sensitive to ACh than the control (triangles, Fig. 4B). Taken together these data illustrate that the leucine insertion in M2 does not prevent assembly nor function of the receptor but provokes an increase of its apparent affinity to ACh.

Leucine insertion reduces the receptor calcium permeability

Recent studies of the effects of nicotine on brain slices have demonstrated the existence of β2 containing neuronal nAChRs in presynaptic buttons (15). These experiments suggest that, given their high calcium permeability, activation of neuronal nAChRs can modulate the release of other neurotransmitters.

To assess the possible influence of the leucine insertion on the nAChR calcium permeability, ionic substitution experiments were designed. To prevent possible contamination of the ACh-evoked current by calcium activated chloride conductance

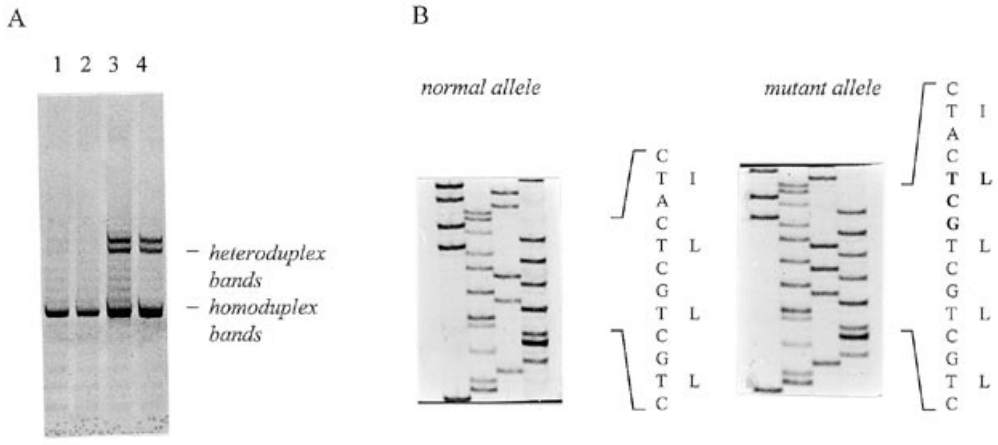

Figure 2. (**A**) Examples of heteroduplex analysis. Lanes 1 and 2, controls; lanes 3 and 4, family members IV1 and IV2. Heteroduplex bands are formed through cross-annealing between normal and mutant DNA strands. Reduced mobility of the reassociated products result in band patterns different from those formed by the homoduplex bands. (**B**) Sequence analysis of the 776ins3 mutation. The normal allele as well as the mutant allele carrying the 3 bp insertion are shown. For the normal and mutant alleles the letter string on the left indicates the DNA base sequence, and the letter string on the right indicates the equivalent amino acid for each base pair triplet.

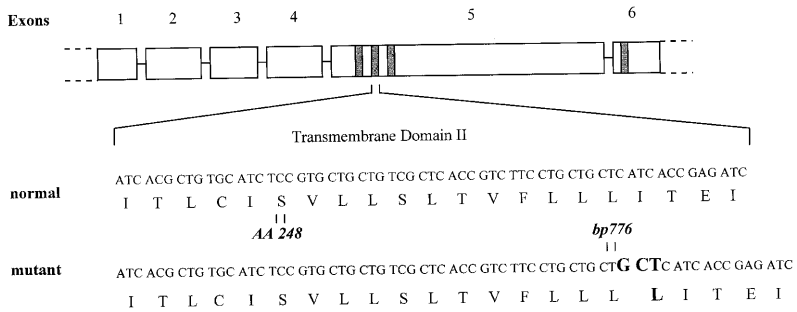

Figure 3. Exon structure of the CHRNA4 gene with parts of the nucleotide and deduced amino acid sequence of the normal and*776ins3*-mutant gene. Transmembrane domains 1–4 are indicated by filled squares. AA248 is the position of the previously detected missense mutation Ser248Phe (5).

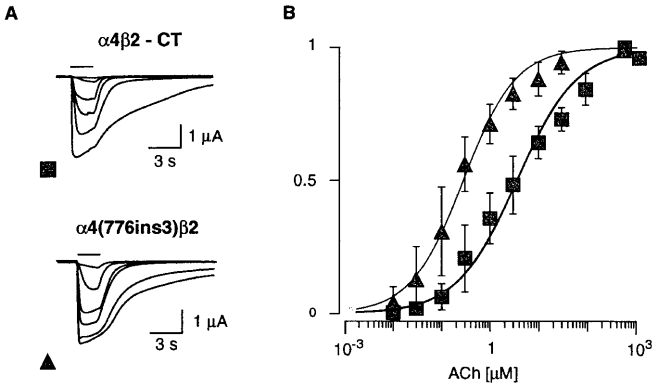

Figure 4. Functional nAChR reconstituted with the α4(776ins3) mutant. (**A**) Currents evoked by short pulses (2 s) of ACh at different concentrations were superimposed. Responses recorded in oocytes expressing the control α4β2 nAChR receptor (CT) are shown in the upper panel. Lower panel illustrates ACh-evoked currents recorded in sibling oocytes injected with the α4(776ins3) and β2 subunits. Horizontal bars correspond to the ACh applications. (**B**) ACh dose-response relationships of the control (squares, $n = 5$, in two batches) and mutated receptors (triangles, $n = 8$ from three batches). Currents evoked by short pulses of ACh applied in growing order were plotted as a function of the agonist concentration. For comparison, currents were normalized to unity at their maximal values and standard errors are indicated by the vertical bars. Continuous lines correspond to best fits obtained with the empirical Hill equation with respective EC_{50}s of 3.6 and 0.28 μM ACh and Hill coefficients of 0.7 and 0.8. Cells were held throughout the experiments at –100 mV.

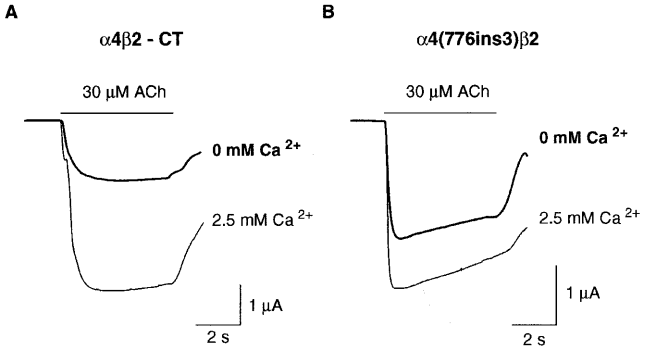

Figure 5. Leucine insertion reduces the α4β2 permeability to calcium. ACh-evoked currents were recorded first in control condition and then in a medium containing no calcium. To prevent contribution of calcium activated chloride channels, oocytes were first injected with 80 nl of a solution containing 10 mM BAPTA. Recordings were performed once no more chloride contribution could be detected from the reversal potential (24). Cells were held in voltage clamp at –100 mV.

oocytes were injected with a potent calcium chelating agent (see Materials and Methods). A few minutes later ACh-evoked currents were recorded first in control and then in a medium deprived of calcium. As shown in Figure 5 this substitution resulted in a large reduction of the ACh-evoked current in the control nAChR (70%, $n = 2$) whereas a much smaller decrease was observed in the mutant receptor (32%, $n = 5$). These data clearly illustrate that insertion of the extra leucine residue at the end of M2 reduces the receptor permeability to calcium.

DISCUSSION

The molecular causes of a number of symptomatic epilepsies, in which seizures are associated with other neurologic deficits, have been identified. These include mutations in mitochondrial DNA, cystatin B and defects causing abnormal neuronal storage, all resulting in neuronal destruction (16–18). In contrast, the molecular lesions underlying the idiopathic epilepsies are likely to lead to more subtle effects on neuronal excitability. In most idiopathic epilepsies the mode of inheritance is complex, and identification of genetic defects is difficult. Uncommon idiopathic single gene epilepsies such as ADNFLE, offer the best chance to identify genes that cause epilepsy as already demonstrated by the Ser248Phe mutation in CHRNA4 in one family (5).

The neuronal nAChR is a pentameric protein composed of varying combinations of α and β subunits. There are at least eight α and three β subunits, with α4 and β2 being the most abundant. The receptor ion channel is lined by the M2 domains of the five subunits. The isolation and cloning of human cDNAs coding for neuronal nAChRs subunits allowed for the first time examination of the receptor properties (14,19). Site directed mutagenesis experiments followed by *in vivo* expression showed that the mutation of a single amino acid in M2 of the nAChR receptor can induce profound modifications of the receptor's physiological and/or pharmacological properties (20–21). With the identification of an association between ADNFLE and a mutation in the CHRNA4 gene [S248F (5)] the necessity of investigation of the receptor's properties became even more evident. Physiological studies of reconstituted nAChRs obtained with this mutant have revealed comparable effects for both the chicken and human nAChRs. The most important effects induced by this

mutation being a significant increase in the receptor desensitization and a reduction of the ACh-evoked current measured at saturation (22–23). Thus, it was concluded that the mutation S248F induces a loss of function of the corresponding α4β2 nAChR that is possibly at the origin of the ADNFLE pathogenesis.

The identification of the insertion of an extra leucine residue at the end of the M2 in the α4 subunit of patients from another family also suffering from ADNFLE could have been predicted to also disrupt receptor function. However, in contrast to this hypothesis, reconstitution experiments performed in *Xenopus* oocytes revealed that functional nAChRs were obtained with the α4(776ins3)β2 constructs. Moreover, no detectable abnormalities were observed at the level of current amplitude nor desensitization between the control and mutant receptors. A significant increase of ~10-fold in the ACh apparent affinity was, however, observed in oocytes expressing the α4(776ins3)β2 nAChR.

Determination of the calcium contribution of the control and mutant receptor revealed that the leucine insertion at the end of M2 induces a significant reduction of the channel permeability for this divalent cation. An observation that correlates rather well with previous findings showing the determinant contribution of leucine 254 and 255 that are at almost equivalent positions in the homomeric α7 nAChR (14). When placed in the light of the most recent findings about the role of presynaptic neuronal nAChR in neurotransmitter release (15,25–26) these data suggest that the leucine insertion results in an indirect loss of function. Thus, although acting by different mechanisms, mutation S248F and the leucine insertion described above can both be considered as rendering the α4β2 nAChR less efficient.

The clinical severity of ADNFLE varies in the Norwegian family and other families. Affected individuals have one normal CHRNA4 gene and one mutant. Thus, in pentameric nAChR incorporating α4 subunits, only some, and perhaps a variable proportion, of M2 domains contribute an extra leucine to the extracellular end of the channel. Varying proportions of defective nAChR could account for variation in clinical severity. The findings in this family confirm that mutations in CHRNA4 are a cause of ADNFLE and demonstrate the presence of allelic heterogeneity. Further studies of the biological effects of these mutations *in vitro* and *in vivo* should allow a better understanding of this human idiopathic epilepsy from molecular lesion to clinical phenotype.

MATERIALS AND METHODS

Subjects

The Norwegian ADNFLE family has been described previously (6). In short, the affected family members had a remarkably consistent clinical presentation. The seizures started in childhood between the ages of 2 and 9 years (median 8 years) and usually remitted in adulthood. The attacks arised only from sleep. Of the eight patients in whom clinical details were available, three were aware of the onset of the seizure because of an aura or because they heard their own vocalisations. The attacks were complex partial seizures of frontal lobe type lasting 15–45 s. The ictus consisted of motor automatisms such as tonic stiffening of the arms, flexion of the knees, clenching of the fists and grimacing with vocal automatisms including shouting, crying, moaning or groaning. There was little or no post-ictal confusion and amnesia for the attacks was usual. Subjects had a normal intellect and a normal neurological examination. The seizures were of varying frequency, often occurring in clusters. Interictal EEGs were normal with the exception of one patient (IV-1) who had bilateral epileptiform discharges in the anterior quadrants. Ictal EEG's were obtained in three cases; one showed only arousal changes but two showed epileptiform activity in the frontal regions.

Individual IV-6 gave a history of mild motor attacks while falling asleep in early teenage years. Her EEG was normal. The clinical history was not sufficiently clear to regard her as definitely affected; she was therefore classified as possibly affected.

Single strand conformation analysis (SSCA) and heteroduplex analysis

Amplification was performed for 32 cycles with an annealing temperature of 66°C. Each PCR was carried out in a 50 µl volume containing 100 ng of genomic DNA, 200 µM of each dNTP, 20 µM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 5% glycerine, 6 pmol of each primer and 1.5 U *Taq* polymerase (Gibco). The primers used for amplification of the CHRNA4 gene were described previously (8). An additional extension cycle (72°C, 10 min) was added at the end of the amplification. For SSCA the PCR products were subsequently denatured for 10 min at 95°C, and stored on ice before electrophoresis through 10% non-denaturing polyacrylamide gels at different temperatures (4°C, RT) was performed. Heteroduplex analysis was carried out after denaturation (95°C, 5 min) and incubation of the PCR products for 30 min at 50°C. The reannealed products were run at 18 V/cm for 2 h at room temperature. After the run, the gels were silver stained and photographed. Fragments showing band shifts were amplified again from genomic DNA using one primer biotinylated at the 5′ end, and single strand sequencing of both alleles using the Dynabeads M-280 Streptavidin-system (Dynal Inc.) was performed. For SSCA band shifts, PCR products were subcloned into pUC18 and transformed into *Escherichia coli*. Colonies carrying the aberrant allele were identified by PCR and subsequent SSCA. Subclones showing band shifts were sequenced.

Cloning of α4 and β2 subunit cDNAs and mutagenesis

The normal α4 and β2 subunit cDNAs have been cloned as described previously (23). For the introduction of the 776ins3 mutation parts of exon 5 of the CHRNA4 gene have been amplified from the genomic DNA of an ADNFLE patient using the following primers: forward primer 5′-CCT GCC CTC CGA GTG TGG C-3′, reverse primer 5′-GCT CGG GCC AGA AGC GCG G-3′. The PCR product was cloned into the *Sma*I site of pUC18 (SureClone kit, Pharmacia) and subclones were screened by SSCA for the presence of the mutant allele. The 776ins3 carrying fragment was excised with *Dra*III/*Mun*I and introduced into the normal α4 cDNA. The cloning constructs were sequenced (Sequenase Version 2.0, Amersham) to verify that only the desired mutation was present.

Electrophysiology

Oocyte preparation, injection and recording were done as previously described (27). Briefly, following a mechanical and enzymatic treatment healthy oocytes were manually selected and intranuclearly injected with 10 nl of solution containing equal

concentrations of α and β cDNAs. Cells were then kept at 18°C and tested 2–4 days later.

Recordings were done using a two electrode voltage clamp (GENECLAMP 500 from Axon Instruments) and data captured on-line on a personal computer using a commercially available interface acquisition card (AT-MIO16 National Instruments). During the experiments oocytes were continuously perfused with control solution and drugs were applied by a fast switching of solution with electrovalves (General Valve, type 3). Acetylcholine (Sigma) was prepared as a stock solution and maintained at –20°C. Drugs were dissolved at final concentration in control medium immediately before the experiments.

To reduce contamination of the ACh-evoked current by the endogenous calcium activated current some experiments were performed after 1,2-bis(2-aminophenoxy)ethane- *N,N,N′,N′*-tetraacetic acid (BAPTA, Fluka) injection. For these experiments a small pipette was filled with BAPTA dissolved in distilled water at a final concentration of 10 mM. Recording electrodes were first inserted into the oocytes and a few minutes later the injection pipette was introduced. A small volume of BAPTA (60–80 nl) was then pressure-injected using the same equipment as for nuclear injection (27).

ACKNOWLEDGEMENTS

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 400/B5) and from the Swiss National Foundation and PNR38 to DB. We thank M.M.Nöthen for providing the DNA of the control individuals.

REFERENCES

1. Scheffer,E.S., Bhatia,K.P., Lopes-Cendes,I., Fish,D.R., Marsden,C.D., Andermann,F., Andermann,E., Desbiens,R., Cendes,F., Manson,J.I. and Berkovic,S.F. (1994) Autosomal dominant nocturnal frontal lobe epilepsy misdiagnosed as sleep disorder. *Lancet*, **343**, 515–517.
2. Scheffer,I.E., Bhatia,K.P., Lopes-Cendes,I., Fish,D.R., Marsden,C.D., Andermann,F., Andermann,E., Desbiens,R., Keene,D., Cendes,F., Manson,J.I., Constantinou,J.E.C., McIntosh,A. and Berkovic,S.F. (1995) Autosomal dominant nocturnal frontal epilepsy: a distinctive clinical disorder. *Brain*, **118**, 61–73.
3. Oldani,A., Zucconi,M., Ferini-Strambi,L., Bizzozero,D. and Smirne,S. (1996) Autosomal dominant nocturnal frontal lobe epilepsy: Electroclinical picture. *Epilepsia*, **37**, 964–976.
4. Phillips,H.A., Scheffer,I.E., Berkovic,S.F., Hollway,G.E., Sutherland,G.R. and Mulley,J.C. (1995) Localization of a gene for autosomal dominant nocturnal frontal lobe epilepsy to chromosome 20q13.2. *Nature Genet.*, **10**, 117–118.
5. Steinlein,O., Mulley,J.C., Propping,P., Wallace,R.H., Phillips,H.A., Sutherland,G.R., Scheffer,I.E. and Berkovic,S.F. (1995) A missense mutation in the neuronal nicotinic acetylcholine receptor α4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nature Genet.*, **11**, 201–203.
6. Magnusson,A., Nakken,K.O. and Brubakk,E. (1996) Autosomal dominant frontal lobe epilepsy. *Lancet*, **347**, 1191–192.
7. Noda,M., Takahashi,H., Tanabe,T., Toyosato,M., Furutani,Y., Hirose,T., Asai,M., Inayama,S., Miyata,T. and Numa,S. (1982) Primary structure of α -subunit precursor of Torpedo californica acetylcholine receptor deduced from cDNA sequence. *Nature*, **299**, 793–823.
8. Steinlein,O., Weiland,S., Stoodt,J. and Propping,P. (1996) Exon-intron structure of the human neuronal nicotinic acetylcholine receptor α4 subunit (CHRNA4). *Genomics*, **32**, 289–294.
9. Ad Hoc Committee on Mutation Nomenclature (1996) Update on nomenclature for human gene mutations. *Hum. Mutat.*, **8**, 197–202.
10. Ballivet,M., Nef,P., Couturier,S., Rungger,D., Bader,C.R., Bertrand,D. and Cooper,E. (1988) Electrophysiology of a chick neuronal nicotinic acetylcholine receptor expressed in *Xenopus* oocytes after cDNA injection. *Neuron*, **1**, 847–852.
11. Bertrand,D., Ballivet,M. and Rungger,D. (1990) Activation and blocking of neuronal nicotinic acetylcholine receptor reconstituted in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA*, **87**, 1993–1997.
12. Buisson,B., Gopalakrishnan,M., Arneric,S.P., Sullivan,J.P. and Bertrand,D. (1996) Human α4 β2 neuronal nicotinic acetylcholine receptor in HEK 293 cells: A patch-clamp study. *J Neurosci.*, **16**, 7880–7891.
13. Deneris,E.S., Connoly,J., Boulter,J., Wada,E., Wada,K., Swanson,L.W., Patrick,J. and Heinemann,S. (1988) Primary structure and expression of β2 : a novel subunit of neuronal nicotinic acetylcholine receptors. *Neuron*, **1**, 45–54.
14. Peng,X., Katz,M., Gerzanich,V., Anand,R. and Lindstrom,J. (1994) Human a7 acetylcholine receptor: cloning of the α7 subunit from the SH-SY5Y cell line and determination of pharmacological properties of native receptors and functional α7 homomers expressed in *Xenopus* oocytes. *Mol. Pharmacol.*, **45**, 546–54.
15. Léna,C. and Changeux,J.P. (1997) Role of Ca^{2+} ions in nicotinic facilitation of GABA release in mouse thalamus. *J. Neurosci.*, **17**, 576–585.
16. Pennacchio,L.A., Lehesjoki,A.E., Stone,N.E., Willour,V.L., Virtaneva,K., Miao,J., D'Amato,E., Ramirez,L., Faham,M., Koskiniemi,M., Warrington,J.A., Norio,R., de la Chapelle,A., Cox,D.R. and Myers,R.M. (1996) Mutations in the gene encoding Cystatin B in progressive myoclonus epilepsy (EPM1). *Science*, **271**, 1731–1734.
17. Wallace,D.C., Zheng,X.X., Lott,M.T., Schoffner,J.M., Hodge,J.A., Kelley,R.I., Epstein,C.M. and Hopkins,L.C. (1988) Familial mitochondrial encephalomyopathy (MERRF): Genetic, pathophysiological, and biochemical characterization of a mitochondrial DNA disease. *Cell*, **55**, 601–605.
18. The International Batten Disease Consortium (1995) Isolation of a novel gene underlying Batten disease. *Cell*, **82**, 949–957.
19. Gopalakrishnan,M., Buisson,B., Touma,E., Giordano,T., Campbell,J.E., Hu,I.C., Donnelyroberts,D., Arneric,S.P., Bertrand,D. and Sullivan,J.P. (1995) Stable expression and pharmacological properties of the human α7 nicotinic acetylcholine receptor. *Eur. J. Pharmacol. Mol. Pharmacol.*, **290**, 237–246.
20. Bertrand,D., Devillers-Thiéry,A., Revah,F., Galzi,J.L., Hussy,N., Mulle,C., Bertrand,S., Ballivet,M. and Changeux,J.P. (1992) Unconventional pharmacology of a neuronal nicotinic receptor mutated in the channel domain. *Proc. Natl. Acad. Sci. USA*, **89**, 1261–1265.
21. Revah,F., Bertrand,D., Galzi,J.L., Devillers,T.A., Mulle,C., Hussy,N., Bertrand,S., Ballivet,M. and Changeux,J.P. (1991) Mutations in the channel domain alter desensitization of a neuronal nicotinic receptor. *Nature*, **353**, 846–849.
22. Buisson,B., Curtis,L. and Bertrand, D. (1997) Neuronal nicotinic acetylcholine receptor and epilepsy. In Genton,P. *et al.* (eds) *Genetics of Focal Epilepsy: Experimental and Clinical Correlates* John Libbey, London, in press.
23. Weiland,S., Witzemann,V., Villarroel,A., Propping,P. and Steinlein,O. (1997) An amino acid exchange in the second transmembrane segment of a neuronal nicotinic receptor causes partial epilepsy by altering its desensitization kinetics. *FEBS Lett.*, **398**, 91–96.
24. Bertrand,D., Galzi,J.L., Deviellers-Thiery,A., Bertrand,S. and Changeux,J.P. (1993) Mutations at two distinct sites within the channel domain M2 alter calcium permeability of neuronal α7 nicotinic receptors. *Proc. Natl. Acad. Sci. USA*, **90**, 6971–6975.
25. Gray,R., Rajan,A.S., Radcliffe,K.A., Yakehiro,M. and Dani,J.A. (1996) Hippocampal synaptic transmission enhanced by low concentrations of nicotine. *Nature*, **383**, 713–716.
26. McGehee,D.S. and Role,L.W. (1996) Neurobiology - Memories of nicotine. *Nature*, **383**, 670–671.
27. Bertrand,D., Cooper,E., Valera,S., Rungger,D. and Ballivet,M. (1991) Electrophysiology of neuronal nicotinic acetylcholine receptors expressed in *Xenopus* oocytes following nuclear injection of genes or cDNA. In Conn,M. (ed.) *Methods in Neuroscience*, New York, pp. 174–193. Academic Press, New York, Boston.